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TITLE: The role of a mitochondrial progesterone receptor in the growth of breast epithelial cells

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14. ABSTRACT This proposal proves a new molecular mechanism whereby progesterone via a mitochondrial receptor, named PR-M, influences the growth of breast cancer cells by enhancing cellular respiration. For this purpose we developed an RNAi assay to silence expression of PR-M in T47D breast cancer cells. We have demonstrated with this assay a decrease in PR-M transcript levels by qRT-PCR and a decrease in protein levels with western blot analysis. Functionally, we then demonstrated a decrease in progesterone/progestin induced mitochondrial membrane potential with silencing of PR-M expression. We then sought to determine the influence of PR-M silencing on the metabolomic pathway of these cells. These studies are still ongoing but initially suggest an increase in lipid catabolism with increased levels of acylcarnitines. These results suggest that progesterone increases cellular energy production by influencing fatty acid oxidation via a unique mitochondrial progesterone receptor. This provides a new mechanism whereby progesterone influences the growth and survival of breast cancer cells.

15. SUBJECT TERMS
mitochondrial progesterone receptor, cellular respiration, apoptosis, T47D cells, RNAi assay

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End-of-Project Summary: DoD Award W81XWH-08-1-0549

This summary describes work accomplished from 9/01/09 to 4/30/2010. Our last report described the following accomplishments.

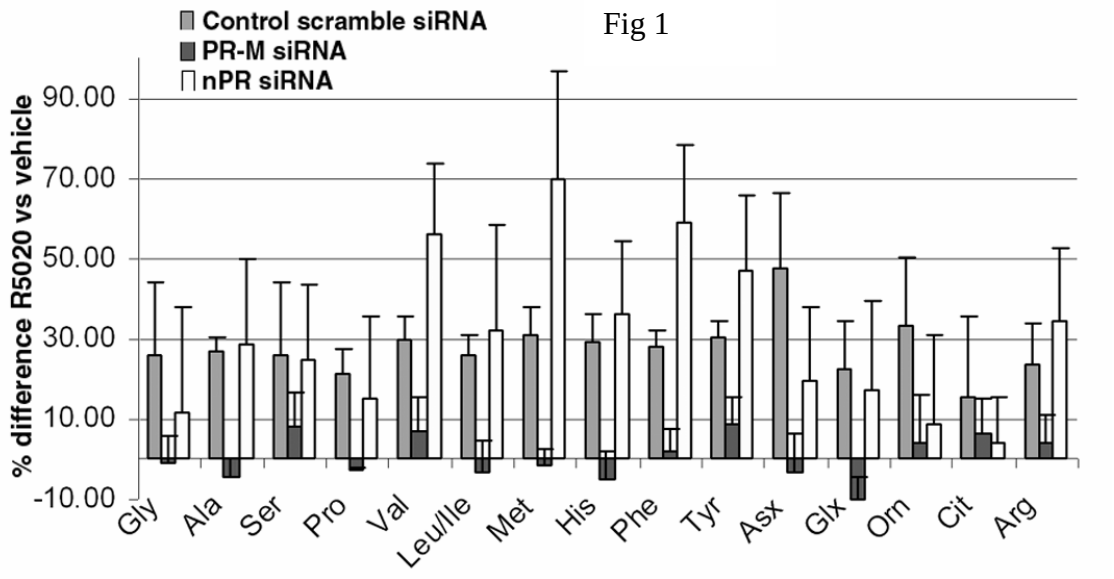
- **Development of a RNAi assay for PR-M in T47D breast cancer cells.**
- **Development of a RNAi assay for nPR in T47D breast cancer cells.**
- **Demonstration that progesterone/progestin induced increase in mitochondrial membrane potential is mediated by PR-M.**

During the no cost extension, we have focused on the following task.

Task 2C: Determine the change in cellular levels of amino acids, acylcarnitines and organic acids in untransfected, mock transfected and siRNA transfected T47D and T47D-Y cells after progesterone treatment, (Month 9-10).

We have completed one set of triplicate experiments investigating amino acid (AA) and acylcarnitine (AC) changes in T47D cells with PR-M knockdown,

Fig 1



nPR knockdown and scrambled siRNA. Cells were treated in media with CSS for 30 min with 10^{-8} M R5020 or vehicle. **Fig 1** shows the % difference (mean ±SEM) of treated versus vehicle in scrambled siRNA, PR-M siRNA and nPR siRNA. An obvious catabolic effect is seen with R5020 treatment in the scrambled siRNA cells with an increase in most amino acids. This effect is obviated by PR-M knockdown, but not affected by nPR knockdown. Statistical analysis showed significant differences between control and PR-M knockdown for Valine, Methionine and Tyrosine.

Fig 2

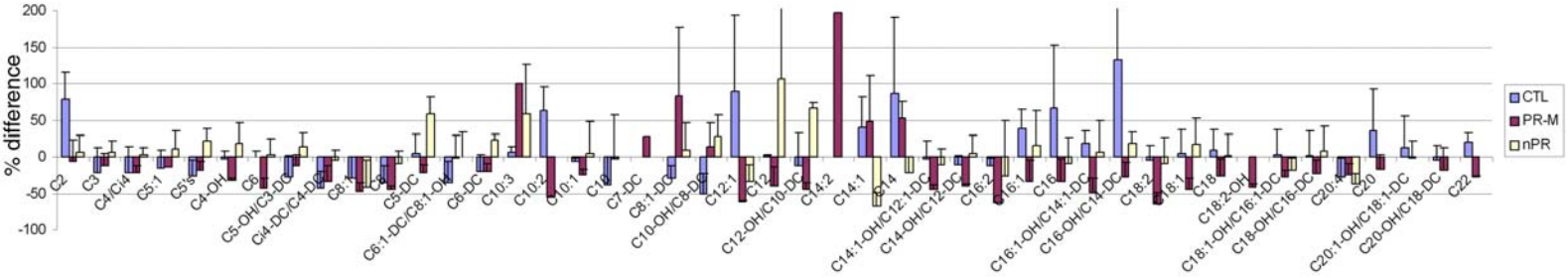


Fig 2 shows the % difference (mean ±SEM) of treated versus vehicle in scrambled siRNA, PR-M siRNA and nPR siRNA for acylcarnitines. As can be seen the deviation was much greater for most of the AC measurements compared to the AA measurements. Thus, the individual AC changes are not significantly different. Yet, a trend can certainly be recognized especially in the longer chain AC of 16 carbons and higher. In this category R5020 treatment in the scrambled siRNA and nPR siRNA transfected cells general resulted in an increase in AC levels. In contrast, blocking PR-M production in the PR-M siRNA transfected cells resulted in a decline in longer chain AC levels with R5020 treatment.

Although these results are preliminary and will need repeating with different doses of the progestin, they strongly suggest a catabolic effect of progestin that is mediated by PR-M.

Summary of accomplishments and discoveries during this grant

1. **Development of a RNAi assay for PR-M in T47D breast cancer cells.**
2. **Development of a RNAi assay for nPR in T47D breast cancer cells.**
3. **Demonstration that progestin induced increase in mitochondrial membrane potential is mediated by PR-M.**
4. **Demonstration that progestin increases amino acid and acylcarnitine levels via PR-M.**

These discoveries lend support to our hypothesis that progesterone increases cellular respiration via catabolic reactions by way of the mitochondrial receptor, PR-M.

REPORTABLE OUTCOMES

- **Results from this grant were presented as an oral presentation at the Annual meeting of the Society of Gynecological Investigation held in Orlando, FA in March 2010.**

- **Results from this grant were used in an R01 NIH application submitted in January, 2010.**
- **A publication including these results is currently in progress.**

FIGURES

Fig 1

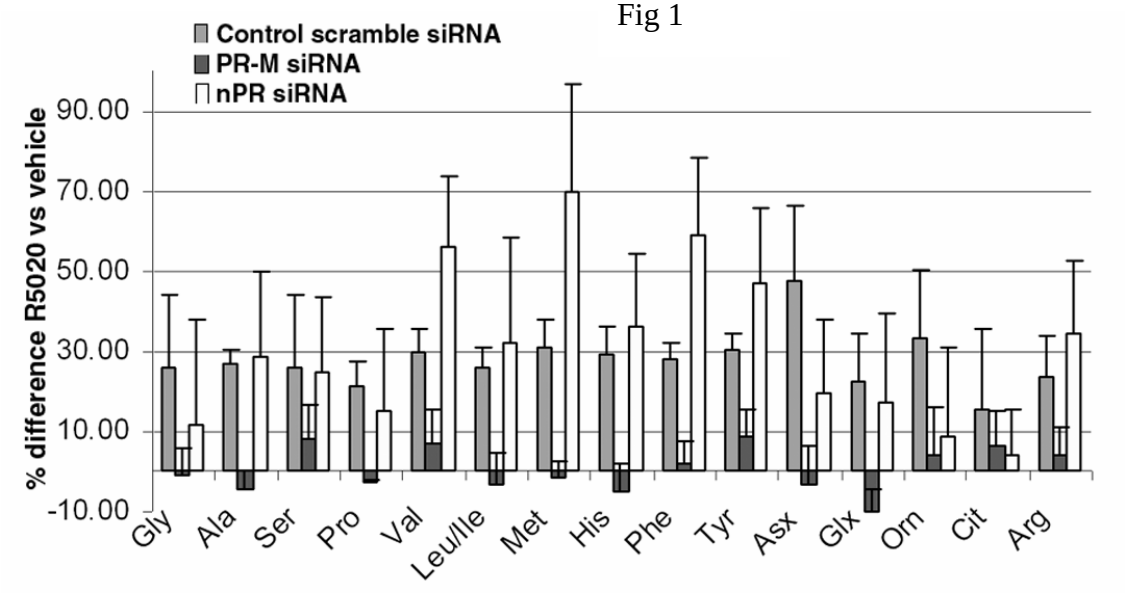


Figure 1: Changes in amino acid levels in triplicate experiemtns after 30 min treatment with 10^{-8} M R5020 in T47D cells transfected with control scrambled siRNA (gray), PR-M targeted siRNA (black) and nPR targeted siRNA (white). Results are expressed as % difference between R5020 treatment and vehicle. Statistical analysis showed significant differences between control and PR-M knockdown for Valine, Methionine and Tyrosine, $P < 0.05$.

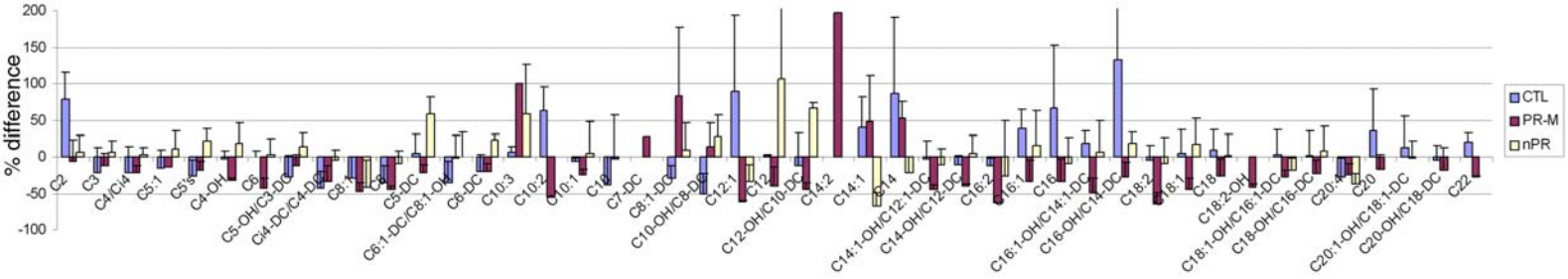


Figure 2: Changes in acylcarnitine levels in triplicate experiemtns after 30 min treatment with 10^{-8} M R5020 in T47D cells transfected with control scrambled siRNA (gray), PR-M targeted siRNA (black) and nPR targeted siRNA (white). Results are expressed as % difference between R5020 treatment and vehicle. No significant differences were seen due to high deviations between the groups.